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FIELD TECHNIQUES FOR STAINING-RECAPTURE EXPERIMENTS WITH COMMERCIAL SHRIMP

by T.J. Costello

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FIELD TECHNIQUES FOR STAINING-RECAPTURE EXPERIMENTS WITH COMMERCIAL SHRIMP

by

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by

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ABSTRACT

Field application of the staining method for marking commercial shrimp is treated in detail. The method is shown to be simple, rapid, and applicable over a wide range of shrimp sizes. It is proving useful for studies of shrimp migration, growth, and mortality. Two disadvantages are: (1) the requirement that, for purposes of measuring growth, stained shrimp be of uniform size when released and (2) the need for developing a high degree of interest among fisherman so that maximum numbers of stained shrimp will be noticed and recovered from commercial catches.

An outline of practical procedures for planning and conducting staining-recapture experiments with shrimp takes into account (1) the scope of the individual experiment relative to the availability and condition of shrimp, (2) field facilities and equipment, (3) kinds of staining agents, manner of application, and precautions to be exercised in their use, and (4) the importance of an intensive recovery program.

INTRODUCTION

Tagging and other marking methods have long been used to identify experimental animals in population and behavior studies. Considerable knowledge about fishes, particularly, has accumulated through application of a wide variety of fish-marking techniques. When properly designed and executed, mark-recapture experiments provide information on fish population movements and measures of population growth and mortality.

Since their exoskeleton is shed when molting occurs, crustaceans are less suited than fishes for the attachment of tags. Despite this shortcoming, several experiments employing tags have yielded valuable information about shrimp populations (Lindner and Anderson, 1956; Iversen and Idyll, 1960). Due to the shrimp's fragility, however, effective application of tags is restricted to those portions of a population comprising the largest and most robust individuals.

Seeking an alternative marking method, Menzel (1955) reported that white shrimp *(Penaeus setiferus)* carefully injected with nontoxic solutions of biological stain, suffered relatively little mortality, and after periods of up to 60 days still retained sufficient stain to permit identification. Moreover, the method appeared feasible for marking the smallest juveniles as well as the largest adults. Dawson (1957) elaborated on Menzel's technique and, experimenting in the laboratory with a wide variety of stains, found four that promised to be suitable for large-scale, mark-recapture experiments. Feeding and immersion were also tested as means for marking shrimp, but the injection technique gave best results. In all methods the primary retention site of the stain proved to be in the branchial region.

The staining technique was first employed at the field level in 1956 by the Bureau of Commercial Fisheries (Costello, 1959). Results were encouraging. A series of experiments that began in 1959 and terminated in 1962 provided basic information on the geographical range of stocks that support the valuable pink shrimp *(Penaeus duorarum)* fishery on the Tortugas grounds (Costello and Allen, 1960).

Based on nearly 4 years' field experience, the present report describes means for circumventing or mitigating difficulties that may be encountered in shrimp staining experiments. It is offered primarily as a guide for agencies contemplating shrimp research in which the staining technique might be incorporated as a tool.

FIELD OPERATIONS

The nature of preparations for an experiment in which vital stains are to be used to mark shrimp depends in large measure on the status of knowledge regarding shrimp populations in the study area as well as on the scope of work to be accomplished. Shrimp populations supporting well-established fisheries lend themselves readily to mark-recapture experiments. If fishery statistics and certain hydrographic data (especially sea-temperature measurements) are also available, their incorporation in analysis of experimental results may be expected to increase the amount of useful information obtained.

General

Staining sites where shrimp can be held under conditions conducive to their survival should be selected. This may require a series of water-quality observations which include checks on dissolved oxygen, turbidity, salinity range, and prevailing daytime and nighttime water temperature. When working with pink shrimp we found that low-salinity (<10 p.p.t.) water should be avoided since they became particularly delicate in such an environment. We also encountered high mortality in these shrimp when the water temperature (surface or bottom) exceeded 75° F. The foregoing does not infer that successful marking experiments are impossible outside the temperature and salinity limits indicated; however, these limits indicate points at which mortality due to handling and marking will rise rapidly. Effective handling of large numbers of pink shrimp outside these limits will require considerably larger holding facilities than are recommended herein.

Marking experiments in offshore areas require that the shrimp be raised rapidly to the surface, marked, and returned to the bottom with minimum injury. This treatment subjects them to a series of physical stresses not yet fully understood. Minimizing exposure to predation upon release also poses a problem. Experience gained by initiating and carrying out mark-recapture experiments under a variety of conditions (for example, in, near, or at some distance from areas at heavy fishing activity; at different seasons; etc.) has indicated that greatest success when using the staining method to estimate mortality will be achieved when releases are made just prior to peak shrimp abundance in the study area.

Other factors to be considered include the expected number and distribution of commercial fishing vessels in the study area and special conditions such as closed seasons. Where regulations restrict shrimp trawling during certain periods, completion of the mark and release phase a short time before the season reopens may be advantageous in allowing desired dispersal before marked shrimp are subject to recapture.

Obtaining Experimental Material

The techniques of catching, transporting, and holding live juvenile shrimp have been well developed by commercial bait fishermen around the Gulf of Mexico (De Sylva, 1954; Woodburn, Eldred, Clark, Hutton, and Ingle, 1957; Inglis and Chin, 1959). When undertaking a marking experiment involving juvenile shrimp, it is convenient to hire an experienced fisherman to obtain suitable quantities of live specimens in good condition and arrange for their transportation to the marking site. Where this is not possible, it will be necessary to

operate some type of fishing device that does not subject the shrimp to too rough treatment during capture. For use in many shallow-water areas, the most effective and versatile gear for this purpose is the bait shrimp "scrape" or roller-frame trawl described by Dumont and Sundstrom (1961). In some localities "channel nets" (fig. 1) may be used to capture young shrimp for experimental purposes. These nets are placed in deep-water channels or passes after dark following a flood tide. Shrimp are caught as they move seaward with the ebbing tide.

For experiments in deeper water offshore, shrimp may be obtained with otter trawls of the type commonly used by the commercial shrimp fleet. Trawling areas should be selected that contain a minimum of dead shells or other heavy material, which may enter the trawl and tend to crush the captured shrimp as they are brought to the surface. Individual drags should be restricted to 10 minutes or less (time on the bottom) to minimize losses due to compression of the catch. Such mortality may be further reduced by sewing into the trawl cod end two 24-inch (diameter) metal spreading rings about 4 feet apart.

Holding and Grading Live Shrimp

Pink shrimp can be held alive and in good condition if crowding is avoided, water temperatures do not exceed 75° F., and the salinity is stabilized at or near that of the water from which they are caught. Feeding should be avoided even when circumstances necessitate extended holding periods.

When working in estuaries or other shallow-water areas, two types of floating cages or "live cars" have been found suitable for holding shrimp during staining operations. One is a plastic 25-gallon garbage can perforated with 1/4-inch holes and supported in the water by an inflated automobile inner tube. The second is a rectangular frame box, 18 inches by 16 inches by 10 inches, constructed of wood and covered with Fiberglas window screening. Either will hold 300 to 500 live shrimp, the number varying with water temperature and shrimp size. In a marking operation, several small cages are preferable to a single large one. They are readily portable and can be easily attached to a dock or vessel.

In offshore operations, special equipment must be provided to maintain shrimp in good

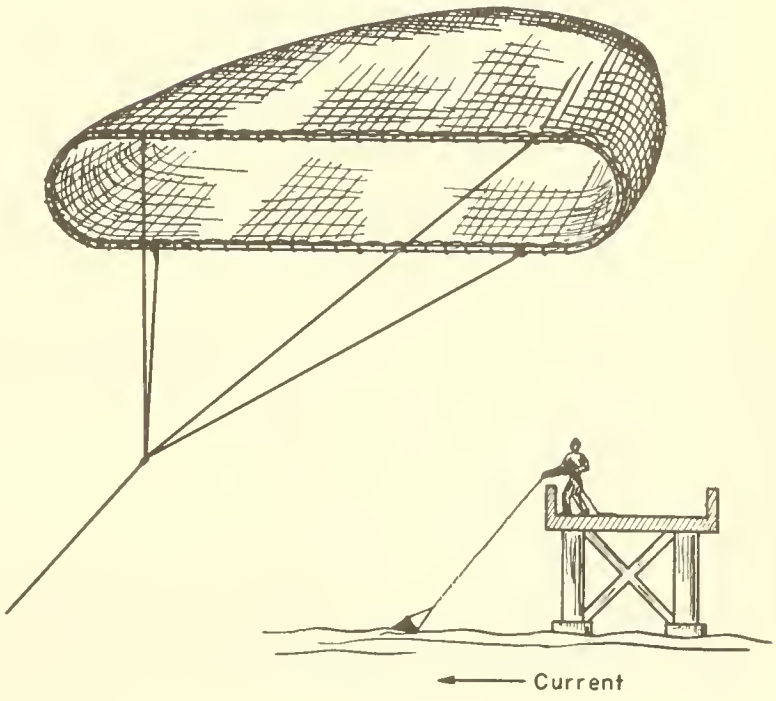

Figure 1.--Channel net used to capture migrating shrimp.

condition. A box found suitable for holding large numbers of live shrimp aboard a vessel is shown in figure 2. Box size may be modified according to available deck space, and it will ordinarily be best to carry two boxes of equal size--one on each side of the vessel to prevent undesirable list. On small vessels where extra weight may create a problem, the amount of water carried in the boxes can be decreased without materially altering the usefulness of the holding box by merely lowering the height of the overflow hole.

All water should be screened before it is piped into holding boxes. A coarse filter placed at the intake and a fine-mesh filter inserted in the water supply line just before it connects with the holding box adequately serve this purpose. With about 15 pounds' pressure, the 1-inch pipe will deliver 6 to 8 gallons per minute of well-aerated sea water through the four siphon filler-drain nozzles (figs. 2 and 3).

If growth rate is to be estimated from stained shrimp recaptures, marked specimens should be of uniform size when released. Allen and Costello (1962) describe a simple yet efficient sorting device adaptable to any field condition.

MASS-MARKING TECHNIQUES

Equipment (Shore-Based)

Field equipment, which facilitates rapid marking of large numbers of shrimp, is shown set up ready for use in figure 4. It consists of lightweight, portable equipment. The water pump is driven by a gasoline engine, so no electrical supply is required. The holding box is of simpler design than that suggested for shipboard use. This box (item M, fig. 4) will hold 3,000-4,000 medium-sized shrimp (total length, 85 to 110 mm.) 6 to 8 hours without excessive mortality.

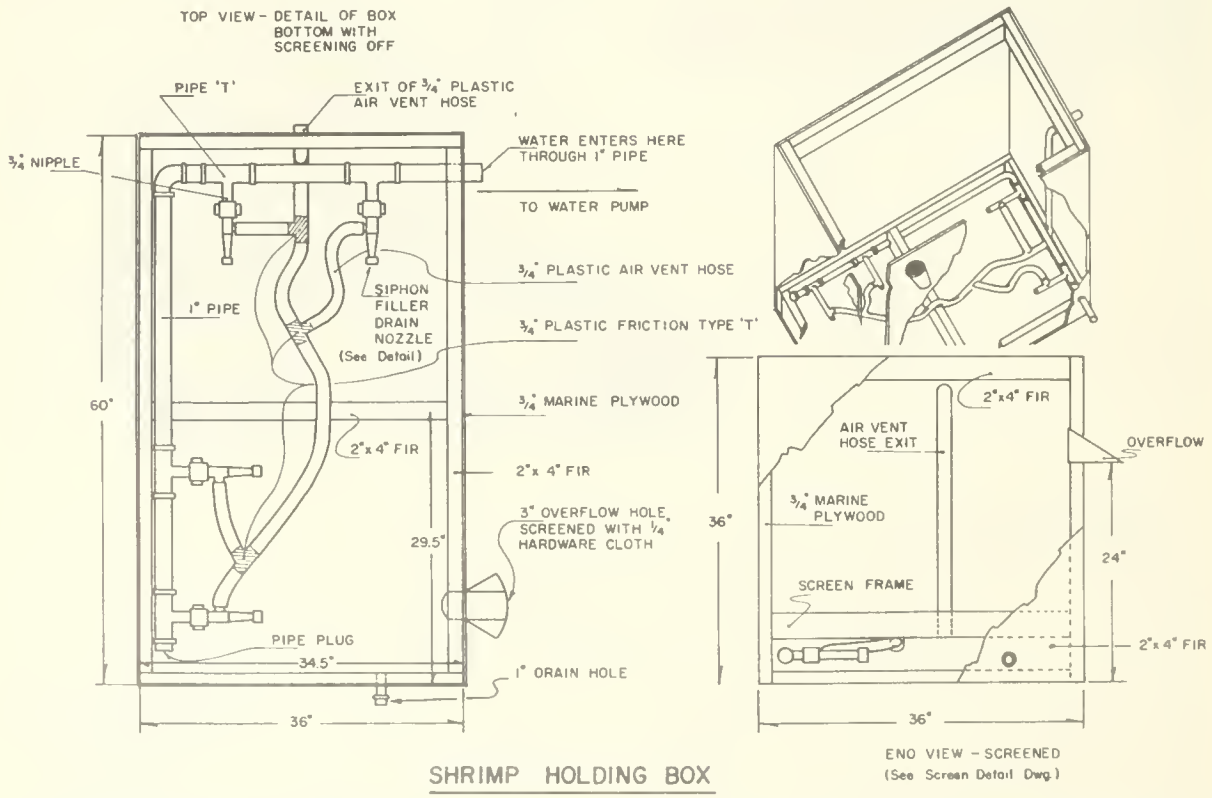

Figure 2.--Shrimp holding box suitable for use at sea. A box of these dimensions will hold 6,000-8,000 shrimp (85 to 110 mm. total length) for several days if the temperature is maintained between 60° and 75° F.

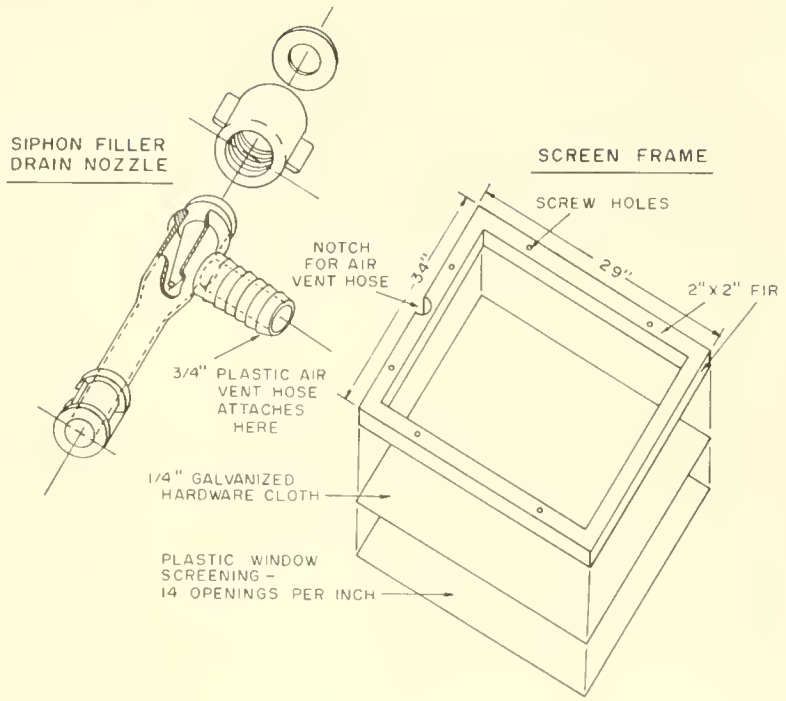

Figure 3.--Detail drawing of a siphon filler-drain nozzle and screen frame for holding box.

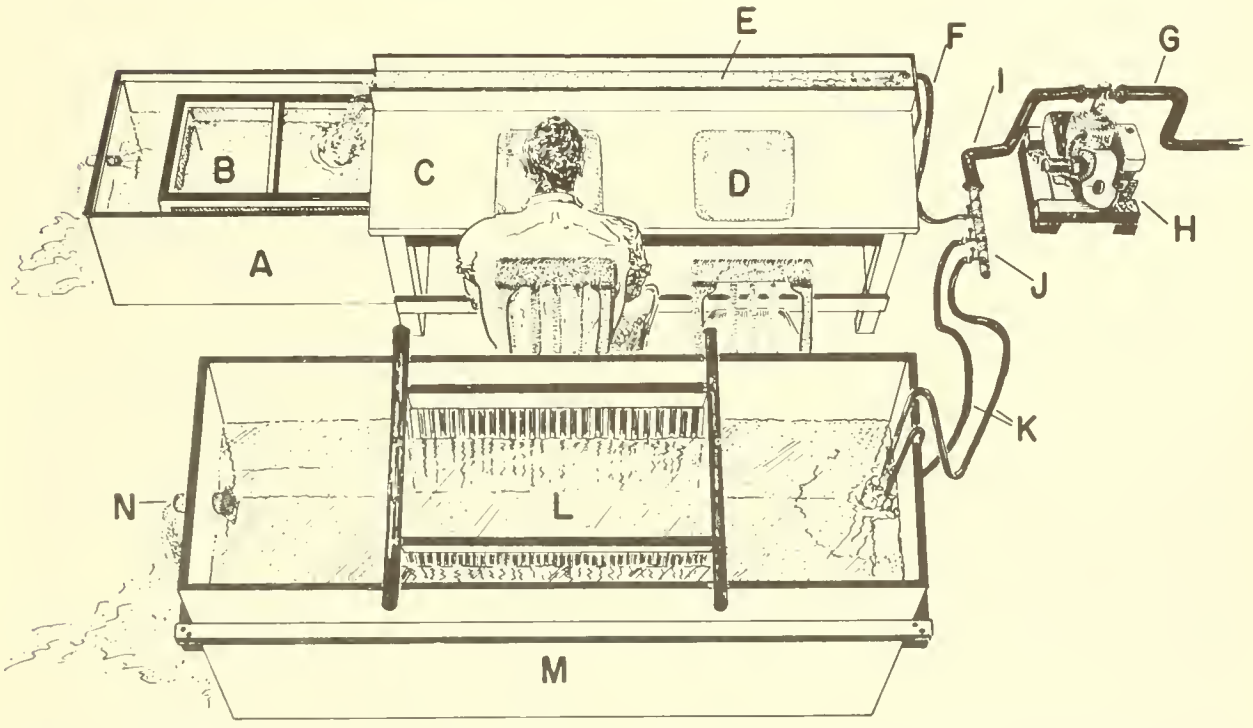

Figure 4.--Suggested arrangement of staining table and holding boxes. (A) Plywood tank, 45 inches by 23 inches by 24 inches. (B) Floating box for holding shrimp after staining. (C) Staining table, 6 feet by 24 inches by 28 inches. (D) Plastic insert tray, 14 inches by 12 inches by 4 inches, holds shrimp to be stained. (E) 4- by 4-inch trough for running water. (F) 3/4-inch hose. (G) 1-1/2-inch intake hose. (H) Water pump. (I) 1-1/2-inch water discharge hose. (J) Pipe connections and gate valves to regulate pressure of water supply. (K) Two 3/4-inch hoses with air bleed on one. (L) Shrimp-grading device. (M) Plywood tank, 6 feet by 30 inches by 32 inches, holds shrimp prior to staining. (N) 3-1/2-inch screened overflow.

Equipment (Shipboard)

Adaptation of shore-based marking equipment for use aboard a vessel involves attaching large items securely to the deck. In inclement weather, baffles should be placed in shrimp-holding boxes to minimize any injury the shrimp might receive from surging water.

Staining Instruments

Tuberculin syringes of 1/2 or 1 cc. capacity and fitted with 1/4-inch hypodermic needles are used for staining. Thirty-gauge needles have proved best for injecting stain into all sizes of shrimp. Small-gauge needles cause little tissue damage at the injection sites. Their use also reduces dripping of low-viscosity staining solutions between injections and measurably speeds the staining process.

Staining Procedure

As live shrimp are received for marking, they are placed in the large holding box (M, fig. 4). If grading is required, it precedes the marking operation. The shrimp are placed in the shrimp-grading device (L) suspended in the large holding box. Ungraded or graded shrimp are dipped, a small number at a time, into the table trays (D). After injection, marked shrimp are tossed into the trough of running sea water (E) where they are carried to holding cage (B).

Stains may be injected into shrimp through the articular membrane of either the fifth or the sixth abdominal joint with the former being the preferred site (fig. 5). Very small shrimp (60 mm. total length or smaller) are more easily marked by injecting the stain laterally through the sclerite of the first abdominal segment (fig. 6).

Dawson's (1957) description of the proper method of handling shrimp for injection is quoted as follows:

> Holding the syringe in the right hand, a shrimp was grasped with the left so that its head was pointed toward the left wrist and with its abdomen held in a flexed position by the left thumb and forefinger. The needle was then introduced through the articular membrane of the sixth abdominal joint slightly to the left of the middorsal line and at an angle approximating 45 degrees. The needle was inserted to a depth of from 2 to 4 millimeters until stain was visibly entering the blood-vascular system through the dorsal abdominal artery.

Figure 5.--Injection through articular membrane of fifth abdominal joint is recommended for shrimp longer than 60 mm. total length.

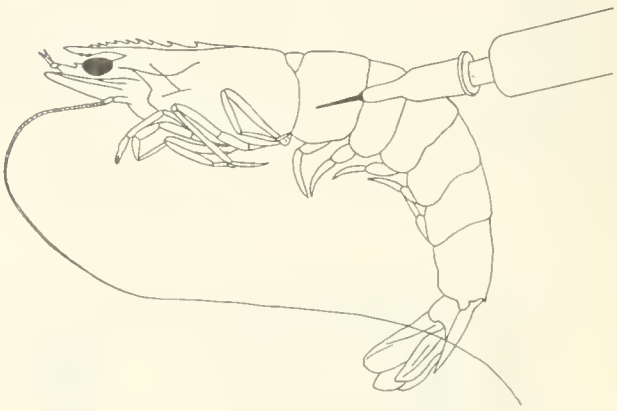

Figure 6.--Injection laterally through sclerite of first abdominal segment is recommended for shrimp shorter than 60 mm. total length.

Care should be taken not to puncture the hindgut when the hypodermic needle is inserted. Also, injection of air in the form of minute bubbles during the staining process will cause shrimp mortality. This problem can be eliminated by holding the syringe and needle in a vertical position (needle up) for a moment after the solution is drawn and forcing out any visible bubbles by slight pressure on the plunger.

Marking Rate

Observations based on several field experiments indicate that an inexperienced person can mark 200 to 240 shrimp per hour; experienced workers can often mark 300 or more shrimp per hour. Marking shrimp by injection is tedious work, so in spite of the rate-per-hour given, one person may be expected to mark no more than 2,000 shrimp a day.

Field experiments require that the number of marked shrimp be counted prior to release. This is best done as the shrimp are being injected. A set of 10 colored beads strung on a small wire serves this purpose very nicely. One bead is moved when 10 shrimp are marked and a 100-tally mark made as the set is completed. Losses due to marking can be determined as the shrimp are released and then subtracted from the total number marked.

RELEASE OF MARKED SHRIMP

The manner of handling shrimp after the dye is injected and until they are safely returned to the water is important. Since shrimp suffer temporary impairment of swimming ability and some loss of equilibrium upon injection, they should not ordinarily be released for at least 4 hours following staining. Because during this period they are also less agile than unmarked animals and react slowly to external stimuli, they are more prone to predation.

In experiments designed to provide an estimate of fishing mortality, it is essential that we know within rather narrow limits the actual number of stained shrimp released alive and in good condition. It is suggested, therefore, that whenever it is feasible to do so, each group of stained shrimp be held for 24 hours following injection. Due to handling and marking there is always a small mortality that will not be apparent in the first 4 hours.

In areas characterized by relatively clear shallow water and active predator populations, marked shrimp should be released a few hundred at a time during daylight hours and observed until they have safely burrowed into the bottom. Rough estimates of immediate losses due to predation may be obtained accordingly. In deep water, releases should be made by use of an underwater release box (fig. 7). The illustrated box has been used successfully to release shrimp on the bottom in water depths to 25 fathoms. In use, the box is attached to a suitable line or cable preferably wound off a power winch. Shrimp are placed in the box after marking, lowered to the bottom slowly, and allowed to escape at or near the bottom when the box-opening device is activated by a weighted messenger.

Marked shrimp being released in the general area where recapture may occur should be liberated in small groups at random so they may mix well with the standing crop. They should ordinarily be released as near as possible to the site of original capture. Rocky bottom areas should be avoided since they frequently contain excessive numbers of predaceous fish.

STAINS AND STAINING SOLUTIONS

Kinds and Quality

Of the four vital dyes reported most useful for marking shrimp (Dawson, 1957), three have been subjected to extensive field and laboratory tests. These are fast green FCF, Trypan blue, and Trypan red. The fourth, Niagara sky blue 6B, has not been field tested. Experience with these dyes indicates that properties of a given dye (color) vary with the manufacturer, which may be due to differences in the mordant characteristic of each product. Culling (1957) points out that difficulty in achieving a satisfactory stain may be experienced when using too pure a form of certain dyes; that is, some impurities are necessary components in many staining compounds.

The fast green FCF manufactured by National Aniline and the Trypan blue and Trypan red manufactured by Harleco proved satisfactory. Trypan blue and fast green FCF both

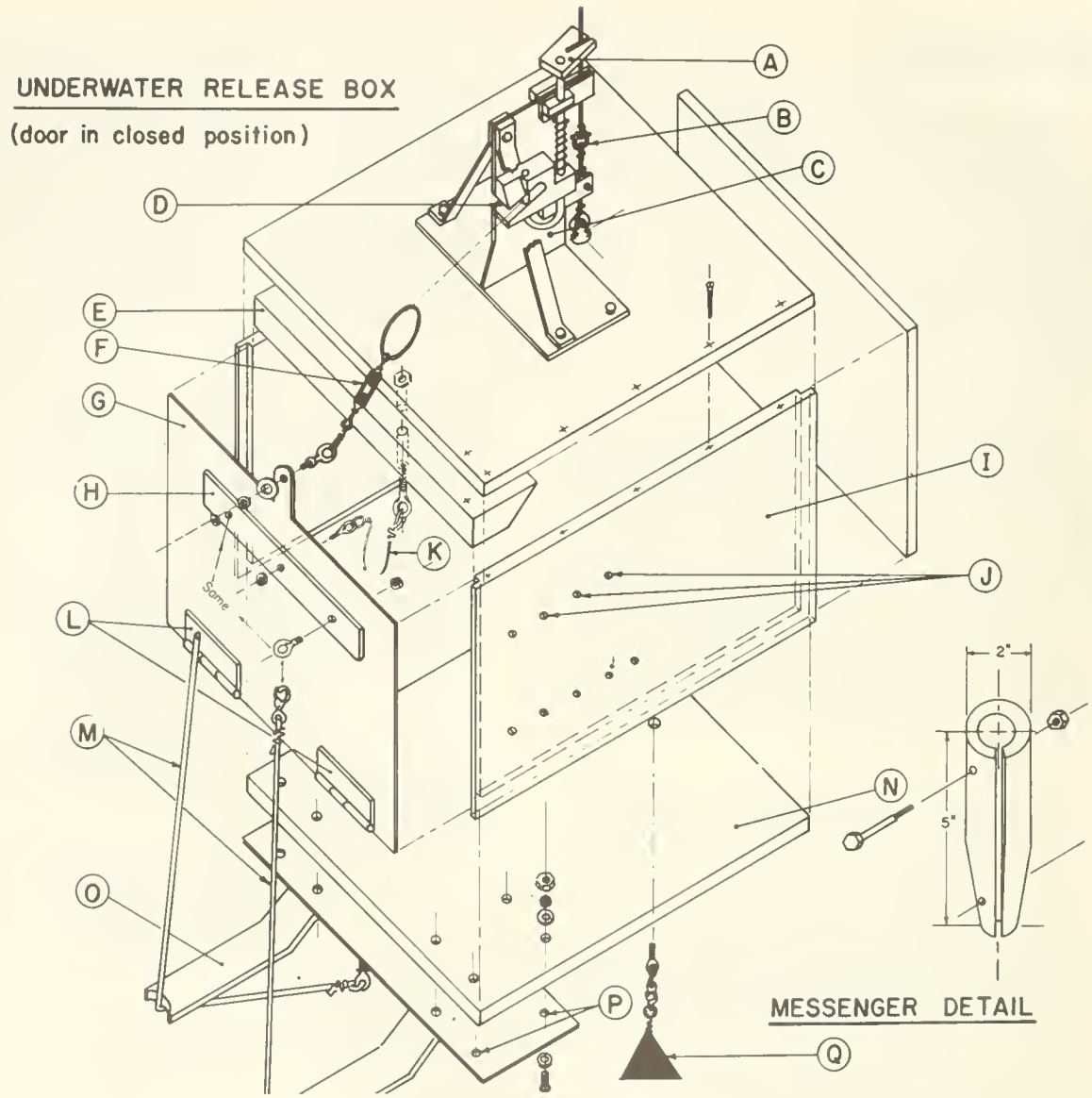

Figure 7.--Underwater release box. (A) Messenger strikes this plate to open door. (B) Shackle bolt (attachment to wire cable at this point). (C) Door-release mechanism (see Kemp, Hardy, and MacKintosh, 1929, for detail drawing of a similar device). (D) Brass ring fits into this slot when box door is closed. (E) Wood block provides base for attaching nylon restraining cord, K, and stop for metal door, G. (F) Turnbuckle, for adjusting length of the linkage which holds door, G, in closed position. (G) Metal door constructed of 1/8-inch steel plate. (H) 4-lb. lead plate gives steel door proper balance for quick opening. (I) 3/4-inch marine plywood. (J) Shows approximate spacing of 1/4-inch holes which should be drilled in all plywood sections of box. (K) Nylon restraining cord limits door opening to 90 position. (L) Metal hinges welded to steel door, G, and to steel plate on box bottom. (M) Surgical rubber tubing (7/16-inch diameter) when stretched, provides the power to open metal door, G. (N) 5/8-inch marine plywood. (O) Metal supports welded to metal plate provide guides for surgical rubber tubing. (P) Holes in metal plate for bolts which attach plate to box bottom. (Q) 12-lb. lead weight aids in sinking box to bottom. Messenger detail: After the release box has reached the bottom, the messenger (5 lb. in weight) is slipped on the cable through the slot opening. Two threaded bolts are then secured before messenger is released.

give distinct, easily recognized markings. Trypan red also gives a clear mark, but it is less distinctive than the other two because the color tends to blend with the natural color of some shrimp. Trypan red may be an excellent stain for white shrimp since it contrasts with the shrimp's natural color. Unlike fast green FCF and Trypan blue, however, it is subject to some fading.

Solvents and Dilution

An important factor in successful shrimp marking is proper handling of stain solutions. Filtered sea water is the stain solvent most often reported in attempts to mark marine animals with vital dyes. Dawson (1957) used distilled water, filtered sea water, glycerine, mineral oil, and alcohol as stain solvents when testing various stains as shrimp-marking agents. In the four stains he reported as the most potentially useful for marking shrimp, a mixture of 90 percent filtered sea water and 10 percent distilled water was used. He concluded, however, that distilled water was the best all-round solvent. Experience with filtered sea water indicates it is generally satisfactory as a solvent for fast green FCF but unsatisfactory for use with Trypan blue, Trypan red, or Niagara sky blue 6B. When solutions with a sea-water base were used, high mortalities frequently occurred with all stains except fast green FCF. Moreover, stains in solutions of natural or artificial sea water did not always give equally distinct marks from specimen to specimen.

Menzel (1955) suggested that isotonic solutions be used in marking shrimp. Experimentation has shown that isotonic solutions are not only unnecessary but, in fact, are inferior to solutions prepared with distilled water. As demonstrated by Williams (1960), body fluids of *Penaeus* shrimp are very nearly isotonic with respect to the surrounding medium, and the animals demonstrate vigorous osmoregulatory capacity. It is therefore reasonable to assume that no ill effects will develop from injections of small quantities of sterile solutions which are hypotonic with respect to body fluids.

Sterile double-distilled water is recommended as the best solvent for all stains used as marking agents. It can be obtained from any pharmaceutical house.

Standardizing Solutions

Conn (1923) suggests that all dye solutions be defined in terms of dilutions of a saturated solution. This seems reasonable although saturation with any given quantity of solvent is not always easily determined, especially when dealing with suspensions and dark-colored dyes.

For marking shrimp, we need only be concerned with a limited number of aqueous stain solutions in concentrations of 0.5 percent or less. All staining solutions reported herein were made by first preparing a 0.5 percent stock solution from dry stain and then making the required dilutions. Recommended stock solutions (0.5 percent) are prepared by dissolving the following amounts of dry stain[1] in 100 ml. of sterile, double-distilled water (65° F.):

Trypan blue (Harleco, Color Index No. 477)	0.67 g.
Trypan red (Harleco, Color Index No. 438)	0.54 g.
Fast green FCF (National Aniline, Color Index No. 42053)	0.56 g.

Stir mixtures until no large particles remain and filter once through Whatman #1 filter paper or equivalent. Unfiltered solutions have been found unsatisfactory for marking shrimp.

The staining action of certain dye solutions is improved by aging (Conn, 1950). Dawson (1957) found, however, that aged solutions offered no advantage, so stains were usually prepared just before use. Experience not only

[1] For Trypan blue, Trypan red, and fast green FCF of the grades discussed herein, actual dye content of the raw material (as specified on container labels) was 79 percent, unknown, and 94 percent, respectively. In the case of materials which specify a different percentage dye composition, appropriate adjustments should be made in calculating the amount of dry material required.

corroborates Dawson's findings but indicates that some dyes, particularly Trypan blue and Trypan red, become increasingly toxic when allowed to stand in solution for extended periods of time. On one occasion a 0.25 percent solution of Trypan blue aged 3 weeks at room temperature caused over 50 percent mortality when used in a regular staining operation. A fresh solution was mixed from the same lot of stain, and mortality was reduced to less than 8 percent. In discussing the use of benzidine dye solutions (including solutions of Trypan blue and Trypan red) as vital dyes, Foot (1950) states that not only are electrolytes (such as sea water) unsafe as solvents, but it is equally dangerous to allow the solutions to age, because the suspended dye particles may become agglutinated and render the solutions highly toxic.[2] This may have been the cause of some high mortalities experienced when using aqueous solutions of the Trypan dyes.

No attempt has been made to determine the rate at which Trypan blue and Trypan red solutions become toxic with age. It is suggested, however, that neither dye be used if it has been in solution more than 3 days. Fast green FCF apparently remains nontoxic in solution over a long period of time. No observations have been made with Niagara sky blue 6B.

Tolerance to Stains

To evaluate the relative toxicity of the above stains, 18 groups of 50 pink shrimp each (all specimens about 95 mm. in total length) were selected and treated with varying amounts of 0.5 percent solutions. Each group was injected with a different amount of the stain solution and held in circulating sea water for 24 hours. In all, six levels ("doses") were tested. As controls, identical groups were injected with equivalent volumes of sterile distilled water and held with the stained animals. The degree of intolerance to increasing amounts of dye was recorded as the difference in survival between the groups injected with stain solutions and those injected with distilled water. The procedure was repeated once. Final values, computed from two trials at each staining level, are plotted in figure 8. Fast green FCF is clearly the least toxic of the three stains tested. Trypan red is more toxic than fast green FCF but noticeably less toxic than Trypan blue.

In addition, four groups of 25 stained shrimp each were held 90 days to determine if any long-term effect could be shown as a result of staining. Results of this experiment indicated that most mortality from staining occurred within the first 24 hours.

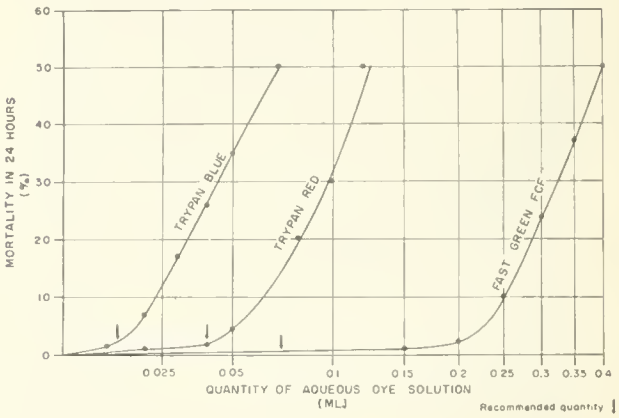

Figure 8.--Tolerance of pink shrimp to injections of varying quantities of three vital dyes in 0.5 percent solution.

Optimum Staining Levels

Experiments with pink shrimp in the size range 70 to 150 mm. total length were conducted to determine the amount of dye required to achieve the most distinct and permanent mark. These shrimp were stained with various solutions ranging in quantity from 0.020 to 0.250 ml. and observed for a 90-day period. Analysis of results yielded the values in table 1, which were found most suitable from the standpoints of minimizing staining mortality and maximizing distinctness and permanency of marks. Similar studies have not been made with brown or white shrimp.

[2] Benzidine dyes do not go into solution when placed in water but remain as colloidal suspensions (Evans and Schulemann, 1914).

Table 1.--Recommended solutions and quantities of three vital dyes used to mark pink shrimp. (Total lengths are midpoints of 20-mm. classes.)

Total length	Trypan blue		Trypan red		Fast green FCF	
	Concentration	Quantity	Concentration	Quantity	Concentration	Quantity
Mm.	*Percent*	*Ml.*	*Percent*	*Ml.*	*Percent*	*Ml.*
75	0.125	0.020	0.250	0.020	0.500	0.030
95	0.250	0.025	0.500	0.040	0.500	0.070
115	0.250	0.035	0.500	0.060	0.500	0.100
135	0.250	0.070	0.500	0.090	0.500	0.180
155	0.500	0.050	0.500	0.140	0.500	0.250

RECOVERING MARKED SHRIMP

The importance of planning each marking experiment so that it provides adequate means for recovery of the marked animals should be stressed. Many workers will agree that a common failing in mark-recapture experiments is that too much attention is given the marking phase and not enough the recovery phase.

Marked shrimp can be readily detected by any perceptive observer and will be returned by fishermen if they know what to look for. In some experiments carried out in Florida, pink shrimp marked with Trypan blue were still recognizable as having been marked more than 10 months after being released. Shrimp marked with fast green FCF have been recaptured, recognized, and returned after more than 6 months since release.

The most practical way to inform fishermen what a marked shrimp looks like is to show them one preserved in a vial of formalin or brine. Samples in vials attached to posters displayed in shrimp processing plants serve almost as well. By providing actual specimens for examination, the likelihood of later receiving discolored but unstained shrimp is greatly reduced. Other means of publicizing experiments as well as descriptions of marked shrimp should also be considered. There is, however, no good substitute for personal contact with individual fishermen.

Rewards should be sufficiently large to induce fishermen and processors to examine catches closely and to return quickly any marked shrimp they may discover. Whenever possible, rewards should be paid promptly and in cash.

SUMMARY

Field observations coupled with the results of laboratory experimentation illustrate the important considerations in successful use of biological dyes for marking shrimp. The staining method is simple, rapid, and is proving useful in studying movement patterns as well as determining growth and mortality rates of commercially important species.

Experience demonstrates that shrimp marked with dyes will be recognized by fishermen, although perhaps not as readily as those marked with tags. Thus, more attention must be given the recovery phase of a staining experiment than that of an experiment in which tags are used.

Important points to consider in the initial phase of a shrimp-staining experiment include condition of water in the area of staining operation, availability of shrimp, and the likelihood of recovering marked specimens. It is also well to check the general condition of the shrimp available for marking, because using many shrimp in soft-shelled condition

could result in excessive marking mortality. A further consideration is that each operation be properly equipped from the standpoint of shrimp-holding devices, staining facilities, etc., and that this equipment be suited to the conditions under which the operation is to be conducted. Probably most important is correct handling of stain solutions. Recommended amounts of stain to be injected into shrimp of various sizes are given as a general guide. Under field conditions, these amounts can only be approximated as staining usually proceeds very rapidly. Of the three vital dyes found most suitable for marking shrimp, fast green FCF and Trypan blue are preferable to Trypan red because they provide a distinctive mark. Fast green FCF is ideally suited for small shrimp because it is noticeably less toxic than the others. Stain solutions should always be freshly prepared and filtered. Sterile, double-distilled water is the recommended stain solvent.

During the staining operation, excessive handling of shrimp and air bubbles in syringes should be avoided. Accurate insertion of the hypodermic needle is essential.

Release of marked shrimp should be delayed at least 4 hours after the stain solution is injected so that specimens not recovering from the shock of handling and staining may be eliminated. Marked specimens should be released in such a way that excessive predation is avoided as they disperse.

ACKNOWLEDGMENT

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LITERATURE CITED

ALLEN, DONALD M., and T. J. COSTELLO.
1962. Grading large numbers of live shrimp for marking experiments. U.S. Fish and Wildlife Service, Progressive Fish-Culturist, vol. 24, no. 1 (January), p. 46-48.

CONN, HAROLD J.
1923. The preparation of staining solutions. Science, vol. 57, p. 15-16.

1950. Stains and staining. In McClung's Handbook of Microscopical Technique, R. M. Jones (editor), 3d ed., rev. & enl., Chapter III, p. 92. Paul B. Hoeber, Inc., New York.

COSTELLO, THOMAS J.
1959. Marking shrimp with biological stains. Proceedings of the Gulf and Caribbean Fisheries Institute, 11th Annual Session, 1958, p. 1-6.

COSTELLO, T. J., and DONALD M. ALLEN.
1960. Notes on the migration and growth of pink shrimp *(Penaeus duorarum)*. Proceedings of the Gulf and Caribbean Fisheries Institute, 12th Annual Session, 1959, p. 5-9.

CULLING, C. F. A.
1957. Handbook of histopathological technique. Butterworth and Co. Publishers Ltd., London, p. 128-137.

DAWSON, C. E.
1957. Studies on marking of commercial shrimp with biological stains. U.S. Fish and Wildlife Service, Special Scientific Report--Fisheries No. 231, 23 p.

DESYLVA, DONALD P.
1954. The live bait shrimp fishery of the northeast coast of Florida. Florida State Board of Conservation Technical Series No. 11, 35 p.

DUMONT, WILLIAM H., and G. T. SUNDSTROM.
1961. Commercial fishing gear of the United States. U.S. Fish and Wildlife Service, Circular 109, p. 36.

EVANS, HERBERT M., and WERNER SCHULEMANN.
1914. The action of vital stains belonging to the benzidine group. Science, vol. 39, p. 443-454.

FOOT, NATHAN CHANDLER.
1950. Vital stains. In McClung's Handbook of Microscopical Technique, R. M. Jones (editor), 3d., rev. & enl., p. 569. Paul B. Hoeber, Inc., New York.

INGLIS, ANTHONY, and EDWARD CHIN.
1959. The bait shrimp industry of the Gulf of Mexico. U.S. Fish and Wildlife Service, Fishery Leaflet 480, 13 p.

IVERSEN, EDWIN S., and C. P. IDYLL.
1960. Aspects of the biology of the Tortugas pink shrimp, *Penaeus duorarum*. Transactions of the American Fisheries Society, vol. 89, no. 1, p. 1-8.

KEMP, STANLEY, A. C. HARDY, and N. A. MACKINTOSH.
1929. Discovery investigations: objects, equipment and methods. Discovery Reports, vol. 1, part 1, p. 193. Cambridge University Press, London.

LINDNER, MILTON J., and WILLIAM W. ANDERSON.
1956. Growth, migrations, spawning and size distribution of shrimp, *Penaeus setiferus*. U.S. Fish and Wildlife Service, Fishery Bulletin 106, vol. 56, p. 553-645.

MENZEL, R. WINSTON.
1955. Marking of shrimp. Science, vol. 121, no. 3143, p. 446.

WILLIAMS, AUSTIN B.
1960. The influence of temperatures on osmotic regulation in two species of estuarine shrimps *(Penaeus)*. Biological Bulletin, vol. 119, no. 3, p. 560-571.

WOODBURN, KENNETH D., BONNIE ELDRED, EUGENIE CLARK, ROBERT F. HUTTON, and ROBERT M. INGLE.
1957. The live bait shrimp industry of the west coast of Florida. Florida State Board of Conservation Technical Series No. 21, 33 p.

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